Multi-scale Modeling of Circadian Rhythms: From Metabolism to Regulation and Back in 24 hours

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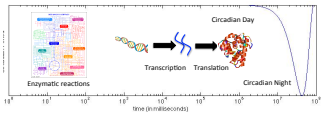
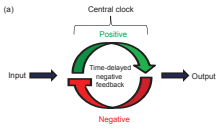
Overview

Goals: The goal of this research is to develop and implement a new computational and theoretical method for modeling biological systems that fills a gap in modeling mass action dynamics. Based on statistical thermodynamics, the method bridges data-poor scales (parameters for mass action kinetics) and data-rich scales (chemical potentials of metabolites, and metabolite, protein & transcript data) to enable predictive modeling from enzymatic reactions (10^{-3} to 10^{0} s^{-1}) to gene and protein regulation (~20 minutes) to circadian rhythms (24 hours). We are:

- Implementing a simulation approach that uses chemical potentials rather than rate constants. This approach involves a rescaling of the fast degrees of freedom, resulting in a compression of the time-dependence to fewer relative scales.
- Understand the relationship between central metabolism and circadian rhythms by using a multi-scale model that includes regulation of the clock.



Circadian Clocks



Circadian clocks lie at the epicenter of cellular physiology for both fungal and mammalian cells, both of which share clocks with conserved regulatory architecture. At the core of these clocks, a heterodimeric transcription factor (TF) drives expression of genes whose protein products feed back, physically interact with, and depress the activity of their heterodimeric activator.

This negative feedback loop, yielding oscillatory TF activity, is the basis of fungal and animal circadian rhythms. Output from the clock occurs when these TFs regulate genes whose products do not impact the core feedback loop. For organisms having a circadian clock, nearly all genes are effectively clock-regulated, yielding the profoundly rhythmic metabolism that has a major impact on adaptation, optimal efficiency of the cell, enzyme production and both normal and disease physiology. *Neurospora crassa* is the best studied cellular circadian system and is a well-established model for eukaryotic, including mammalian, clocks. *Neurospora* provides an extremely tractable system in which to pioneer modeling of these cellular clocks and their influence on metabolism.

Prediction of Dynamics & Regulation

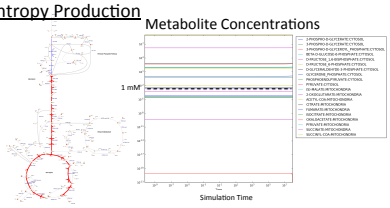
The new approach to the law of mass action does not require rate parameters but instead uses chemical potentials (1). Due to the statistical formulation of the theory, the approach can directly integrate metabolomics and proteomics data.

Simulation Procedure

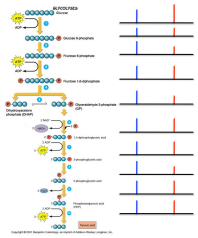
◆ Optimization and Maximum Entropy Production

$$\frac{dn_i}{dt} = \sum_{\alpha}^{Z} c_{\alpha} \frac{1}{\gamma_{i,\alpha}} \left(e^{A_{\alpha}(n_i)/RT} - e^{-A_{\alpha}(n_i)/RT} \right)$$

The Marcelin Equation sets each of the decay rates of the forward and reverse forces to the same rate. Assuming the same decay rate for *all* reactions removes any kinetic bottlenecks in phase space of the system such that the dynamics are governed only by the thermodynamics



◆ Prediction of Regulation



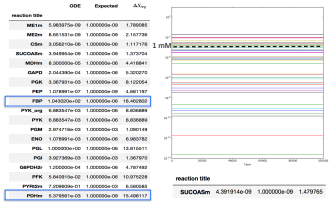
Experiment
Simulation

How similar is the simulation to experimen measurement when averaged over the experimental data?

$$\Delta S_{reg} = \sum_{j}^{k} \rho_j \log\left(\frac{\theta_j}{\rho_j}\right)$$

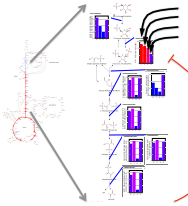
Metabolite Concentrations

Before Regulation | After Regulation



◆ Thermo-Kinetic Simulations

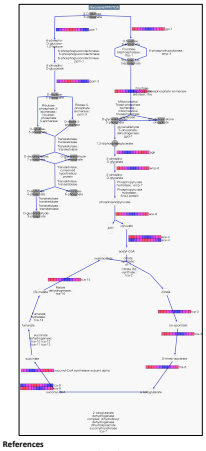
Prediction of energetics of glycolysis reactions from first principles. Columns from left to right indicate: (1) free energy change, (2) power, (3) resistance, and (4) flux. Red indicates high values and blue indicates low values. Units are arbitrary. The phosphofructokinase reaction has dramatically different characteristics than the other reactions because feedback regulation of ATP turns it into a potentiometer.

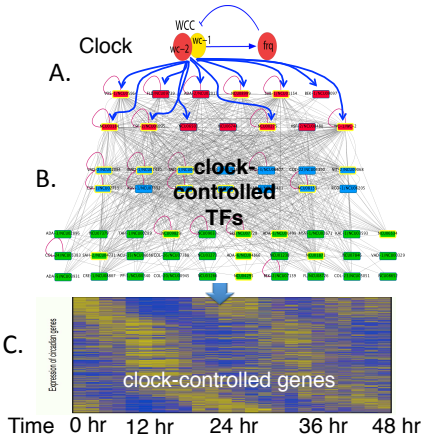


Observation of Dynamics & Regulation

Lower Right: The circadian cycle is approximated in (**A**) by the negative feedback loop in which the heterodimer WC-1/WC-2 drives expression of *frq* which feeds back with other proteins (not shown) to depress WC-1/WC-2 activity. In (**B**), WC-1/WC-2, in turn, activate clock-controlled TFs (curved blue arrows) and these in turn regulate additional TFs, in all comprising a hierarchical network downstream from the clock. This transcriptional network, now largely described from ChIP-seq data for over 50 TFs, acts as a dynamic filter for time information generated by the circadian oscillator in (**A**). In the aggregate the TFs within this transcriptional network act on downstream genes in a combinatorial manner to regulate their expression. Shown in (**C**) is the heat map showing rhythmic expression of the *Neurospora* genome as determined by RNA-seq of samples collected every 2 hrs over 48 hrs in constant darkness.

Lower Left: Environmental controls are known to extensively modulate cellular metabolism in fungi and lead to pre-translational regulation as well [2]. Our work has found that the clock extensively regulates metabolism via protein expression and may, in turn, be regulated by metabolism itself. Our initial assessment of the expression of central metabolism enzymes suggests that glycolysis and the TCA cycle, generating ATP and NADH, are most active during the circadian dusk, while the pentose phosphate pathway, generating NADPH, is most active during the circadian dawn (right, protein expression indicated by heatmaps). Yet to be determined is whether metabolism oscillates independently between glycolysis and the pentose phosphate pathways, or whether metabolism is controlled by regulation of expression at the protein level [3].





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